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(54) Title: METHOD OF TREATING A FOOD PRODUCT

(57) Abstract: A method of treating a food product which includes contacting the food product with an aqueous solution containing about 2 % hydrogen peroxide for a time period of about 60 seconds or greater, wherein the aqueous solution has a temperature of about 50 °C.

METHOD OF TREATING A FOOD PRODUCT

Technical Field of the Invention

The present invention relates generally to a method of treating a food product, and more particularly to a method of treating lettuce.

Background of the Invention

Leafy green vegetables are arguably the foundation of the vegetable industry. For example, lettuces such as leaf and romaine are basic ingredients in vegetable-based salads, Iceberg lettuce in addition to being a basic ingredient in vegetable-based salads also supplies a garnish for sandwiches, and cabbage is the basic ingredient in coleslaw.

Consumption of leafy green vegetables has been trending higher over the past two decades. Leafy green vegetables accounted for about $2.5 billion or 16 percent of all farm cash receipts for vegetables in 1996, up from 13 percent ($1.1 billion) in 1986. Lettuces of all types account for the largest share of farm cash receipts for leafy green vegetables, amounting to more than half in 1996.

One possible reason for the above described popularity of lettuces may include the success of prepackaged salads which provide consumers with a great deal of convenience. However, a concern associated with the consumption of lettuces, such as prepackaged lettuce, is that these vegetables can harbor various pathogens. For example, pathogens such as *E. coli* O157:H7, *Listeria monocytogenes*, and *Shigella sonnei* have been detected on lettuce. To minimize the risk of microbial infection, suitable prevention or decontamination methods are

needed. Several different washing procedures in the fresh produce industry have been used to decontaminate fruits and vegetables. For example, washing with cold chlorinated water is the most widely used method for decontaminating fruits and vegetables. However, the efficacy of chlorine washing is minimal when organic matter is present. Several alternative methods for decontaminating fruits and vegetables have been studied, however most of these methods adversely affect the sensory quality of the lettuce. Accordingly, there is a need for a treatment method of decontaminating a food product, such as lettuce, which addresses one or more of the aforementioned concerns.

Summary of the Invention

In accordance with one embodiment of the 5present invention, there is provided a method of preparing a food product. The method includes contacting the food product with an aqueous solution containing about 2% hydrogen peroxide for a time period of about 60 seconds or greater, wherein the aqueous solution has a temperature of about 50°C.

In accordance with another embodiment of the present invention, there is provided a method for treating a vegetable. The method includes contacting the vegetable with a solution containing about 2% hydrogen peroxide for a time period of about 60 seconds to about 90 seconds, wherein the aqueous solution has a temperature of about 50°C.

In accordance with still another embodiment of the present invention, there is provided a method of treating lettuce. The method includes (a) providing an

aqueous solution which has a temperature of about 50°C and includes about 2% hydrogen peroxide and (b) placing the lettuce in contact with the aqueous solution for a time period of about 60 seconds to about 90 seconds.

In accordance with yet another embodiment of the present invention there is provided method of treating lettuce. The method includes (a) placing the lettuce in contact with a solution (i) having a temperature of about 50°C and (ii) consisting essentially of water and about 2% hydrogen peroxide and (b) placing the lettuce in contact with the solution for a time period of about 60 seconds or greater.

It is an object of the present invention to provide a new and useful method of preparing a food product.

It is another object of the present invention to provide an improved method of preparing a food product.

It is yet another object of the present invention to provide a new and useful method for treating a vegetable.

It is still another object of the present invention to provide an improved method for treating a vegetable.

It is yet another object of the present invention to provide a new and useful method of treating lettuce.

It is still another object of the present invention to provide an improved method of treating lettuce.

It is yet another object of the present invention to provide a method of treating a food product, such as lettuce, which increases its microbiological safety and preserves its sensory quality.

The above and other objects, features, and advantages of the present invention will become apparent from the following description and the attached drawings.

Brief Description of the Drawings

FIG. 1. is a flow chart of an exemplary embodiment of the present invention;

FIG. 2 is a histogram of data from Rep. 1 depicting mean ratings for appearance of treated and untreated pre-cut/packaged lettuce stored for 3, 10, and 15 days at 5°C;

FIG. 3 is a histogram of data from Rep. 2 depicting mean ratings for appearance of treated and untreated pre-cut/packaged lettuce stored for 3, 10, and 15 days at 5°C;

FIG. 4 is a histogram of data from Rep. 1 depicting mean ratings for overall liking of treated and untreated pre-cut/packaged lettuce stored for 3, 10, and 15 days at 5°C;

FIG. 5 is a histogram of data from Rep. 2 depicting mean ratings for overall liking of treated and untreated pre-cut/packaged lettuce stored for 3, 10, and 15 days at 5°C;

FIG. 6 is a histogram of data from Rep. 1 depicting mean ratings for color of treated and untreated pre-cut/ packaged lettuce stored for 3, 10, and 15 days at 5°C;

FIG. 7 is a histogram of data from Rep. 1 depicting mean ratings for aroma of treated and untreated pre-cut/ packaged lettuce stored for 3, 10, and 15 days at 5°C;

FIG. 8 is a histogram of data from Rep. 1 depicting mean ratings for texture of treated and untreated pre-cut/ packaged lettuce stored for 3, 10, and 15 days at 5°C;

FIG. 9 is a histogram of data from Rep. 1 depicting mean ratings for flavor of treated and untreated pre-cut/ packaged lettuce stored for 3, 10, and 15 days at 5°C;

FIG. 10 is a histogram of data from Rep. 2 depicting mean ratings for color of treated and untreated pre-cut/ packaged lettuce stored for 3, 10, and 15 days at 5°C;

FIG. 11 is a histogram of data from Rep. 2 depicting mean ratings for aroma of treated and untreated pre-cut/ packaged lettuce stored for 3, 10, and 15 days at 5°C;

FIG. 12 is a histogram of data from Rep. 2 depicting mean ratings for texture of treated and untreated pre-cut/ packaged lettuce stored for 3, 10, and 15 days at 5°C;

FIG. 13 is a histogram of data from Rep. 2 depicting mean ratings for flavor of treated and untreated pre-cut/ packaged lettuce stored for 3, 10, and 15 days at 5°C; and

FIG. 14 is a representation of a container with lettuce disposed therein.

Detailed Description of a Preferred Embodiment

While the invention is susceptible to various modifications and alternative forms, a specific embodiment thereof has been shown by way of example in the drawings and will herein be described in detail. It should be understood, however, that there is no intent to limit the invention to the particular form disclosed, but on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the appended claims.

In one exemplary embodiment the present invention pertains to methods of treating or preparing a food product which preserves the food product and increases its microbiological safety. Preferably, the food product being treated with a method of the present invention is an uncooked vegetable which has been cut into a number of pieces. More preferably, the food product being treated with a method of the present invention is a member of the Asteraceae family, for example Asparagus lettuce, Bibb lettuce, Boston lettuce, Butterhead lettuce, Celtuce, Cos, Crisphead lettuce, Curled lettuce, Garden lettuce, Green oak-leaf lettuce, Green romaine lettuce, Head lettuce, Iceberg lettuce, Lettuce, Limestone lettuce, Lolla rossa, Loose-leaf lettuce, Perella Red, Red oak-leaf lettuce, Red romaine lettuce, Romaine lettuce, and Tango lettuce.

Now referring to FIG. 1, there is shown a flowchart of one embodiment of the method 10 of the present invention. The method 10 begins with step 12 in which

the food product is cut into a number of pieces. For example, a food product, such as lettuce, is cut or chopped into a number of pieces prior to step 14.

Next, in step 14, the food product is placed in contact with an aqueous solution having a temperature of about 50° C and containing about 2% hydrogen peroxide (H_2O_2) for a time period of about 60 seconds or greater. Preferably, the food product is placed in contact with the aforementioned aqueous solution for a time period of about 60 seconds to about 90 seconds. More preferably, the food product is placed in contact with the aforementioned aqueous solution for a time period of about 60 seconds or about 90 seconds. Suitable techniques for contacting the food product with the aqueous solution include bathing or immersing the food product in the aqueous solution.

It should be appreciated that, as discussed in greater detail below, placing the food product in contact with the aforementioned aqueous solution in the above described manner increases the microbiological safety of the food product while preserving its sensory quality. In particular, it has been discovered that the cooperation between a temperature of about 50° C, the presence of about 2% hydrogen peroxide, and a time period of about 60 seconds to about 90 seconds functions to increase the microbiological safety of a food product while preserving its sensory quality. This is in contrast to other methods of treating a food product which subject a food product to other combinations of physical and/or chemical conditions that do not cooperate to effectively preserve the sensory quality of the food product while increasing its microbiological safety. Furthermore, it should be appreciated that the present invention is particularly useful for food products, such as lettuce,

which are particularly sensitive to being treated with chemicals, and thus can have their sensory quality significantly decreased as a result of being subjected to a treatment to increase their microbiological safety.

Another problem encountered with other methods of treating food products is that some do not kill the pathogenic bacteria present on the food product, but only function to kill nonpathogenic bacteria. This can exacerbate the microbiological safety problem by decreasing the competition faced by the pathogenic bacteria and thus allowing them to flourish to an even greater extent on the food product. Thus, decreasing the microbiological competition faced by the pathogenic bacteria can increase a consumer's risk of contracting a foodborne microbial infection. However, as demonstrated in greater detail below, subjecting a food product to a method of the present invention ensures that pathogenic bacteria present on the food product are inactivated.

Still referring to FIG. 1, after step 14, the food product is removed from the aqueous solution in step 16 and then cooled to a temperature of about 2° C to about 4° C for about ten minutes in step 18. It should be appreciated that, as shown in step 20 of FIG. 1, the food product should be rinsed and/or dewatered following step 18 to substantially remove water containing H_2O_2 from the food product. For example, lettuce (3 lbs) can be centrifuged for 2 minutes in a hand salad spinner at 90 rpm to substantially remove water containing H_2O_2 therefrom.

Now referring to FIG. 14, it is contemplated that after treating the food product with the aforementioned aqueous solution in the above described manner it can be placed into a void 20 defined by a wall 22 of a container 24. For example, as

shown in FIG. 14, after being treated as described above lettuce 26 can be placed into void 20 defined by wall 22 of container 24. Preferably, wall 22 of container 24 has a gas permeability so as to maintain a moderate level of oxygen transfer from container 24. Examples of containers which can be utilized in the present invention include, but are not limited to, those described in United States Patents 6,294,210 B1, 5,840,807, 6,060,136, and 6,086,967 all of which are incorporated herein by reference.

The following discussion is directed to the inactivation of pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* on lettuce by a method of the present invention.

MATERIALS AND METHODS

Bacterial culture. Five strains each of *E. coli* O157:H7 (E06, E08, E10, E16, E22), *S.* Enteritidis (E565-88, E-180-88, E-457-88, SE61696, E-1294), and *L. monocytogenes* (LM109, LM116, LM201, LM ATCC 19117, LM H7550 serotype 4b) were used. The bacterial strains were cultured individually in 10 ml of tryptic soy broth (TSB, Difco Laboratories, Detroit, MI) three successive 24-hr intervals at 37°C. After incubation, each culture was centrifuged twice at 4000 X *g* for 10 minutes, then washed and resuspended in 10 ml of sterile 0.1% peptone water. For each pathogen, equal portions of each bacterial strains were combined and used as the inoculum (about 10^8 CFU/ml). The bacterial count of the five-strain mixture of each pathogen was determined by plating 0.1-ml portions of appropriate dilutions on

duplicate tryptic soy agar plates (TSA, Difco Laboratories), and incubating the plates at 37°C for 24 h.

Inoculation and antimicrobial treatments. Iceberg lettuce was purchased from a grocery store and stored at 4°C on the day before the experiment. On the day of the experiment, two to three layers of outside leaves were discarded. Intact inside leaves were removed and cut into 11.5 x 8 cm pieces. The lettuce leaves were placed in a laminar hood and one hundred µl of bacterial suspension was delivered in ten drops on the surface of a lettuce leaf. Bacterial suspensions on the lettuce leaves were dried in the laminar hood for 45 to 55 minutes at room temperature. After drying, each inoculated leaf was placed into a quart-size Ziploc® bag (Johnson & Son, Inc., Racine WI). To obtain a baseline, 200 ml of 0.1% peptone water were added to the Ziploc® bag, then agitated on an orbital shaker at 300 rpm for 2 minutes. Lettuce leaves were treated with about 2% hydrogen peroxide at about 50°C for about 60 seconds or about 90 seconds. Lettuce leaves treated with sterile deionized water under the same conditions were used for control samples. Two hundred ml of fresh-made treatment solutions or sterile deionized water were added into the Ziploc® bag which was immersed into a water bath at experiment temperature. Control samples were tested side-by-side with treated samples. The treatment solution and deionized water were warmed up to the treatment temperature in the water bath before the experiment. Three leaves were used for each treatment and duplicate trials were tested for each treatment condition and bacterial species.

Enumeration of bacterial population. After treatment, the lettuce leaf was individually transferred into a second Ziploc® bag containing 200 ml of Dey-Engley (DE) broth. The bags containing lettuce leaves and DE broth were agitated on an orbital shaker at 300 rpm for 2 minutes. The DE broth in the bags was serially (1:10) diluted in 0.1% peptone water. For undiluted samples, quadruplicate 0.25 ml and duplicate 0.1 ml samples of DE broth were plated. Duplicate 0.1-ml portions of diluted samples were also plated. All plates were incubated at 37°C for 24 h. A volume of 5 ml of undiluted DE broth was transferred to a 250-ml Erlenmeyer flask containing 45 ml of TSB. Following enrichment for 24 h at 37°C, the culture for each flask was streaked onto two selective plates. The pH values of treatment solution, deionized water and DE broth were measured with a pH meter (Accumet Basic, Fisher Scientific; Pittsburg, Pennsylvania).

Bacterial media and confirmation. All media were purchased from Difco Co. (Detroit, MI). Xylose lysine desoxycholate (XLD), Sorbitol MacConkey agar (SMA) and modified Oxford agar (MOX) were used as selective plates for *S.* Enteritidis, *E. coli* O157:H7 and *L. monocytogenes*, respectively. TSA was used as a non-selective media to recover injured bacterial cells. TSB was used as a general enrichment broth for all three bacterial species. Colonies with typical characteristics on selective plates were confirmed serologically by Oxoid (Ogdensburg, NY) latex agglutination assays for *E. coli* O157:H7 and *S. Enteritidis*, or biochemically by API strip (bioMerieux; Hazelwood, MI.) for *L. monocytogenes.*

Appearance of lettuce leaves and hydrogen peroxide residue. Uninoculated lettuce leaves were used to determine sensory quality after the treatment of about

2% hydrogen peroxide at about 50°C. Both exposure times, i.e. about 60 seconds and about 90 seconds, were tested. After being cut and treated as previously described, lettuce leaves were washed by cold tap water at 16 to 20°C for 10 minutes. The lettuce leaves were dried on layers of paper towels, then centrifuged by a hand salad spinner for 2 minutes. Three hundred grams of cut lettuce leaves were packed into a Ziploc® bag and stored at 4°C. Appearance of lettuce leaves was observed on 3, 5, 7, 10 and 15 days after treatment. Residue of hydrogen peroxide on lettuce leaves was determined by the Reflectoquant system (EM Science Inc., Gibbstown, NJ) with a minimum level of 2 ppm. The residue detection was tested immediately after centrifugation and the days of appearance observation.

Statistical analysis. Data from independent replicate trials were pooled, and the mean value and standard deviation were determined by Microsoft Excel 97 for each pathogen and vegetable combination. Significance of difference between treatments was determined by *t* test using the Statistical Analysis System (SAS) program.

RESULTS

Antibacterial activities. Treatment of lettuce with about 2% H_2O_2 at about 50°C for about 90 seconds. reduced *S.* Enteritidis, *E. coli* O157:H7 and *L. monocytogenes* populations by 4.5, 4.7 and 2.7 $\log_{10}$ CFU/leaf, respectively. Similar results were obtained with about 60 seconds of exposure time (see Tables 1 to 3 below). There was no significant difference ($p>0.05$) between the 60 and 90 second treatments.

Table 1. Populations ($\log_{10}$ CFU/leaf) of *S.* Enteritidis on lettuce treated with about 2% H_2O_2 (treated) or deionized water (control) at about 50°C for about 60 seconds or about 90 seconds.

	Exposure Time	
TSA Plates	60 sec	90 sec
Untreated lettuce	7.66±0.33	7.67±0.32
Treated lettuce	3.95±0.41	3.18±0.45
Control lettuce	6.67±0.37	7.11±0.53
Selective agar plates		
Untreated lettuce	7.59±0.32	7.25±0.38
Treated lettuce	3.68±0.57	2.98±0.24
Control lettuce	6.64±0.40	6.70±0.25

Data were obtained from duplicate trials. Each trial had three samples.
There was no statistically significant difference ($P > 0.05$) in pathogen counts between 60 and 90 second treatments.

Table 2. Populations of *E. coli* O157:H7 on lettuce treated with about 2% H_2O_2 (treated) or deionized water (control) at about 50°C for about 60 seconds or about 90 seconds.

	Exposure Time	
TSA Plates	60 sec	90 sec
Untreated lettuce	7.75±0.40	7.68±0.54
Treated lettuce	3.38±0.74	3.11±0.38
Control lettuce	6.26±0.53	6.85±0.13
Selective agar plates		
Untreated lettuce	7.54±0.32	7.11±0.18
Treated lettuce	2.94±1.06	2.82±0.23
Control lettuce	6.15±0.46	6.05±0.68

Data were obtained from duplicate trials. Each trial had three samples.
There was no statistically significant difference ($P > 0.05$) in pathogen counts between 60 and 90 second treatments.

Table 3. Populations of *L. monocytogenes* on lettuce treated with about 2% H_2O_2 (treated) or deionized water (control) at about 50°C for about 60 seconds or about 90 seconds.

	Exposure Time	
TSA Plates	60 sec	90 sec
Untreated lettuce	7.27±0.26	7.26±0.44
Treated lettuce	4.76±0.01	4.53±0.48
Control lettuce	6.48±0.40	6.96±0.53
Selective agar plates		
Untreated lettuce	6.96±0.18	7.06±0.64
Treated lettuce	4.39±0.06	4.10±0.21
Control lettuce	6.22±0.40	6.23±0.64

Data were obtained from duplicate trials. Each trial had three samples.
There was no statistically significant difference ($P > 0.05$) in pathogen counts between 60 and 90 second treatments.

Appearance of lettuce leaves and hydrogen peroxide residue. Treated and control lettuce leaves were slightly brown after 10 days of storage at about 4°C.

Control lettuce showed more browning than treated samples. All lettuce leaves retained their firmness even after 15 days of storage. Similar results were obtained for lettuce treated for about 90 seconds or about 60 seconds. Results of lettuce appearance are summarized in Table 4 below.

Table 4. The appearance of lettuce leaves treated with about 2% H_2O_2 or deionized water at about 50°C for about 60 seconds or about 90 seconds.

Treatment /Day	Day 3	Day 5	Day 7	Day 10	Day 15
Treated	no browning	no browning	no browning	slight browning on cutting edge	slight browning
Deionized water	no browning	no browning	no browning	slight browning	slight browning

Results were obtained from duplicate trials. Each had three samples for each treatment.

The pH values of about 2% hydrogen peroxide, deionized Water and, DE broth are listed in Table 7 below. Similar pH values of DE broth after treatment were obtained from all treatment solutions. It suggested that buffering capacity of DE broth was enough to neutralize acidity of the combination of lactic acid plus hydrogen peroxide or hydrogen peroxide only. Hydrogen peroxide residue was undetectable immediately after centrifugation.

Table 5. pH values of about 2% H_2O_2 and deionized water before and after treatment. DE broth pH values are shown under treatment solutions.

Treatment	2% H_2O_2	deionized water
before	5.61±0.12	6.61±0.52
after	5.75±0.22	6.51±0.62
DE broth	8.22±0.32	8.31±0.32

Results were obtained from duplicate trials. Each had three samples for each treatment.

The following discussion is directed to the sensory quality of lettuce treated with a method of the present invention.

Materials and Methods

Lettuce and treatment: Iceberg lettuce heads were purchased from a grocery store in Griffin, GA. The store wrapping and two outer leaves were removed. For each batch, 6 heads (about 6 lbs) of lettuce were cored and cut into pieces simulating the sizes currently found in retail packages of salad mixes. Cut pieces (about 3 lbs) were placed in a mesh bag (41 x 54 cm). For the control, two bags containing 3 lbs each were immersed at the same time in a large stainless steel kettle which contained tap water and 6 mesh bags of crushed ice to maintain the water temperature at 4°C. Lettuce was rinsed for 10 minutes in running tap water with the kettle drain open slightly to permit constant outward flow of water. The rinse water was discarded after each batch.

For treated lettuce, the same procedure was followed except that the bags of cut lettuce were immersed for about 60 seconds in about a 2% hydrogen peroxide solution (about 3 L of 30% hydrogen peroxide in about 42 L water) maintained in a steam kettle at 50±2° C. Following treatment, bags were transferred immediately to a kettle containing ice water at about 4° C for a 10-minute rinse.

Lettuce was removed from the mesh bags and poured into a 5-gal manually operated salad spin dryer (Model 811899, Superior Products Mfg. Co., St. Paul, MN); spin time was 3 minutes. Following dewatering, lettuce was added to plastic

containers with lids and held in a 5° C walk-in cooler until packaged a short time later.

Lettuce was packaged in polyethylene film provided by Tanimura and Antle, Inc., Salinas, CA, and by the protocol currently used by the fresh cut industry; 3 lb bags were flushed with O_2 and sealed. Package volume was controlled at 6.0 L, and sealed packages had 10-12% residual O_2. The volume of the package filled with lettuce was measured by placing the package in a container of known volume. The container was then filled with soybean seeds such that the lettuce package was completely surrounded by the seeds. The volume of the package was estimated using the following equation:

$$V_p = V_c - V_s = V_c - W_s/D_s$$

where,

V_c = Volume of container (l)

V_s = Volume of seeds (l)

V_p = Volume of package (l)

D_s = Bulk density of seeds (gm/l)

W_s = Weight of seeds (gm)

Headspace composition was verified by gas chromatographic (GC) analysis. A Hewlett Packard GC (Model 5890) equipped with thermal conductivity detector and 182.9 cm long CRT-I column (Alltech Associates, Deerfield, IL) and operated as follows: oven 40° C; injector 120° C; detector 250° C; and N_2 flow rate, 20 l/hr. Packaged lettuce was held at 5° C and evaluated after 3, 10 and 15 days of storage.

Panelists: Forty panelists were recruited from the local community to evaluate Rep. 1 on April 26, 2001; another 40 were recruited to evaluate Rep. 2 on May 3, 2001. Criteria for participation were that consumers (1) be at least 18 years of age, (2) be the primary shopper for the household, (3) purchase and eat lettuce regularly, and (4) not be allergic to lettuce or hydrogen peroxide.

Test procedure: Five sensory panels with eight panelists per session were conducted on two days at 1:00, 2:00, 4:30, 5:30 and 6:30 p.m. When consumers arrived, they were asked to fill out two consent forms approved by the University of Georgia Institutional Review Board, an honorarium form, and a demographic questionnaire. During orientation, panelists were instructed on the evaluation procedures and use of the signal lights in the sensory booths. Panelists were seated in individual partitioned booths in the climate-controlled sensory evaluation laboratory. Each booth was illuminated with incandescent lighting (473 lux).

On the morning of the sensory test, bags were removed from storage and headspace gas composition determined by GC analysis. The bags were then opened and the lettuce poured into a large bowl. Obviously discolored/defective pieces were discarded, as food preparers would. Samples were filled into 8-oz plastic cups labeled with 3-digit code numbers, covered with snap-on lids, and refrigerated until served that afternoon. Six samples (3 controls and 3 treated for each storage time) were presented one at the time in a random sequence. Panelists evaluated appearance and color first, then aroma, flavor, texture, and overall liking using a 9-point hedonic scale with 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely. They also indicated whether the sample was

acceptable (yes or no response). Space was provided on the ballot for the panelists to comment freely about the samples. Water and unsalted crackers were provided for cleansing the palate between samples. Covered expectoration cups were provided if the panelists did not wish to swallow the samples. After the sensory evaluation, panelists completed a questionnaire which dealt with lettuce purchasing, handling, and storing practices and willingness-to-buy attitudes. Panelists received a $10 honorarium for their participation.

Instrumental color measurements: Lettuce color was measured using a Gardner colorimeter (model XL-845, Pacific Scientific, Bethesda, MD) standardized against a green reference tile. Sufficient sample was placed in a black sample cup (91 mm diameter) to completely cover the bottom. The lid was placed on the cup, and the Hunter color readings (L^*, a^*, b^*) were recorded. Four readings per sample were obtained by rotating the cup at 90° angles. Psychometric color terms of chroma (intensity or saturation), hue angle, and total color difference (ΔE) were calculated from values of L^* (lightness), $+a^*$ (redness), $-a^*$ (greenness), $+b^*$ (yellowness), and $-b^*$ (blueness).

Statistical analysis: Sensory ratings and instrumental color data were analyzed using the General Linear Model (GLM) procedure. Means and frequencies were obtained using the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Significant means were separated using the Least Significant Difference (LSD) test.

Results and Discussion

Demographic characteristics: Because the demographic characteristics of the two groups of panelists who participated on April 26 and May 3 were similar, they were combined and are shown below in Table 6.

Table 6. Demographic characteristics of participants in sensory evaluation of pre-cut/packaged lettuce, Spring, 2001.

Characteristic	Percentage
Age	
18-34 yrs	19.5
35-44 yrs	23.4
45-54 yrs	10.4
55-64 yrs	28.5
65-75 yrs or older	18.2
	(n = 77)
Gender	
Male	23.4
Female	76.6
	(n = 77)
Race	
White	84.4
Black	10.4
Asian	5.2
	(n = 77)
Marital Status	
Never married	6.5
Married	72.7
Separated/Divorced	9.1
Widowed	11.7
	(n = 77)
Educational Level	
Less than 8 years of school	1.3
9-12 yrs of school	11.7
Completed high school or equivalent	29.9
Completed vocational school or some college	36.3
Completed college	7.8
Graduate or professional school	13.0
	(n = 77)
Employment Status	
Employed full-time	42.8
Employed part-time	7.8
Unemployed	20.8
Retired	28.6
	(n = 77)
Household Income Before Taxes	
$19,999 or less	21.6
$20,000 to $39,999	45.8
$40,000 to $59,999	16.3
$60,000 and over	16.3
	(n = 74)

The age of the consumers was distributed relatively evenly among categories. Panelists were primarily female (77%), white (84%), and married (73%). Educational levels ranged from 13% with 12 yrs of school or less, 30% having completed high school, 36% having completed vocational school or some college, 8% having completed college and 13% having completed graduate or professional school. About 43% were employed full-time, and 29% were retired. About 46% had a household income before taxes in the $20,000 to $39,999 range.

Package Volume and Headspace Gas Composition: Mean and standard deviations of initial package volume, and oxygen and carbon dioxide content at the beginning and end of storage time are presented in Table 7 below.

Table 7. Mean and standard deviation values for package volume and headspace gas compositions of lettuce stored at 5° C.

Sample	Package volume (liters)	Headspace gas composition (%)			
		Initial		Final	
		O_2	CO_2	O_2	CO_2
REP 1					
Mean	6.30	10.39	0.08	0.44	7.24
Standard deviation	0.41	0.16	0.04	0.16	2.17
REP 2					
Mean	5.98	10.37	0.08	0.44	5.89
Standard deviation	0.18	0.13	0.04	0.15	1.12

As noted earlier, all packages were not stored for the same number of days; however, no particular trend was observed in change in gas composition due to storage. Thus, overall mean and standard deviation values of final gas composition (at the end of storage) were calculated and reported in Table 7. Data in Table 7

confirms that a reasonable control on the desired initial volume (~ 6 L) and oxygen gas content (~10%) was maintained. It was observed that there was a quick drop in oxygen content and increase of carbon dioxide values due to high respiration of cut lettuce. The packages were not stored for more than 15 days, but holding of these packages for extended periods of time at low oxygen and carbon dioxide content may lead to growth of anaerobic microorganisms.

Instrumental color measurements: Mean values for instrumental color measurements of pre-cut/packaged control and treated lettuce stored for 3, 10, and 15 days at 5° C are shown below in Table 8.

Table 8. Mean values for instrumental color measurements of pre-cut/packaged control and treated lettuce stored for 3, 10, and 15 days at 5° C.

Sample	L*	a*	b*	Chroma	Hue Angle	ΔE
Rep. 1						
Control, day 3	65.9 ab	-10.2	24.1	26.1	113.0	17.2 abc
Treated, day 3	67.1 a	-9.4	24.1	25.9	111.0	18.0 ab
Control, day 10	60.8 bc	-7.4	19.2	20.6	110.8	14.5 d
Treated, day 10	61.9 ab	-10.6	21.9	24.3	115.9	15.3 cd
Control, day 15	62.3 ab	-9.5	22.1	24.1	113.0	15.6 bcd
Treated, day 15	55.6 c	-8.2	22.1	23.6	110.5	18.5 a
Pr > F	0.0106	0.3927	0.1761	0.2420	0.2906	0.0304
Rep. 2						
Control, day 3	66.4 a	-15.1a	24.1	28.4	122.0 c	16.6
Treated, day 3	66.8 a	-17.6b	25.9	31.3	124.3 ab	18.2
Control, day 10	65.5 a	-14.8a	24.3	28.5	121.5 c	16.3
Treated, day 10	65.5 a	-15.3a	23.7	28.2	122.9 bc	15.7
Control, day 15	62.8 ab	-16.1ab	24.3	29.2	123.3 bc	16.5
Treated, day 15	59.5 b	-17.4b	23.5	29.3	126.4 a	16.6
Pr > F	0.0222	0.0220	0.4078	0.2676	0.0020	0.4023

[1] Values in a column, within a Rep., not followed by the same letter are significantly different ($p \leq 0.05$).

[2] L* = lightness (0 = black, 100 = white). Chroma = $(a^{*2} + b^{*2})^{1/2}$. Hue angle = $\tan^{-1}(b^*/a^*)$. Total color difference (ΔE) = $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

The two replications were not pooled because some of the color characteristics at the two test dates were statistically different ($p \leq 0.05$), particularly with regard to greenness (-a* values) and hue angle. In Rep. 1 (April 26 test date), significant treatment effects were noted in lightness (L*) and total color difference (ΔE), whereas for Rep. 2 (May 3 test date), lightness, greenness and hue angle were

affected by treatment. In Rep. 1, the treated/15 day stored lettuce was the darkest (lowest L* value) and most different in overall color (highest ΔE value) than the other samples. In Rep. 2, this sample was also the darkest and had a greener hue (higher hue angle) than the other samples.

Sensory Evaluation: Replication differences were also observed in the sensory data; therefore, reps were not pooled for the two test dates. Results for appearance are shown in Figure 2 for Rep. 1 and in Figure 3 for Rep. 2. Results for overall liking are shown in Figure 4 for Rep. 1 and in Figure 5 for Rep. 2. Similar trends were observed for color (see FIG. 6 for Rep. 1 and FIG. 10 for Rep. 2), aroma (see FIG. 7 for Rep. 1 and FIG. 11 for Rep. 2), texture (see FIG. FIG. 8 for Rep. 1 and FIG. 12 for Rep. 2), and flavor (see FIG. 9 for Rep. 1 and FIG. 13 for Rep. 2). It is assumed that the greener the color of lettuce leaves, the fresher it was perceived to be by consumers. As noted in the instrumental color data, the lettuce evaluated on April 26 was considerably less green than that evaluated on May 3. This color difference influenced the participants' perceptions of lettuce quality and acceptability. For example, in lettuce that was less green (lower -a* values, Rep. 1, April data), there was no difference between control and treated samples at either day 3 or day 15 for each sensory attribute. For treated samples, there was no difference in hedonic ratings between day 3 and day 10, whereas for controls, there was a marked decrease from day 3 to day 10.

In contrast, when lettuce had more green color (higher -a* values) as was the case for Rep 2 (May data), sensory ratings were never below 4.4. Hedonic ratings for each sensory attribute were consistently higher for treated samples than for

controls at day 3 and day 15 of Rep 2; at day 10, however, hedonic ratings of control and treated lettuce were similar. Overall, the antibacterial treatment was more effective in maintaining sensory quality during the 15-day storage, compared to untreated controls, provided that the lettuce had considerable green color initially.

In response to the question, "Is this product acceptable?", panelists who evaluated Rep. 1 rated both control and treated lettuce at day 3 and treated lettuce at day 10 to be the most acceptable (see Table 9 below).

Table 9. Mean ratings for acceptability of pre-cut/packaged control and treated lettuce stored for 3, 10 and 15 days at 5° C[1].

Sample	Rep. 1	Rep. 2
Control, day 3	1.03 c	1.16 cd
Treated, day 3	1.11 c	1.03 d
Control, day 10	1.49 b	1.38 b
Treated, day 10	1.11 c	1.31 bc
Control, day 15	1.87 a	1.62 a
Treated, day 15	1.81 a	1.23 bc

[1] Rating of 1 = yes, 2 = no. Values in a column not followed by the same letter are different at $p \leq 0.05$.

Samples which were least acceptable were both control and treated lettuce stored for 15 days. For Rep. 2, control and treated samples at day 3 were also rated most acceptable whereas control lettuce at day 15 was least acceptable.

Lettuce purchasing, handling, and storing practices and willingness-to-buy attitudes: Information obtained about consumers' lettuce purchasing, handling, and storing practices and willingness-to-buy attitudes is reported below in Table 10.

Table 10. Lettuce purchasing, handling, storing, and willingness-to-buy characteristics of consumers who participated in lettuce quality evaluation, Spring, 2001 (n = 77).

Questions	Percentage
1. How often do you buy lettuce? (n = 76)	
Less than once a week	46.0
Once a week	40.8
Twice a week	7.9
Three or more times a week	5.3
2. How often do you eat lettuce? (n = 76)	
More than once a day	2.6
Once a day	7.9
Five to six times a week	4.0
Three to four times a week	27.6
One to two times a week	44.7
Less than once a week	13.2
3. What variety of lettuce do you purchase most often? (n = 74)	
Iceberg	81.1
Romaine	10.8
Leaf	6.8
Bibb	1.3
4. Which do you purchase most often? (n = 77)	
Whole head	54.5
Pre-cut/packaged	44.2
Both whole and pre-cut	1.3
5. Do you wash pre-cut/packaged lettuce purchased in the grocery store? (n = 76)	
Yes	47.4
No	52.6
6. Do you drain washed lettuce? (n = 37)	
Yes	94.6
No	5.4

Table 10. *Continued*

Questions		Percentage
7.	If yes, for how long? (n = 36)	
	Less than 5 minutes	58.4
	5 to 10 minutes	25.0
	10 to 20 minutes	8.3
	More than 20 minutes	8.3
8.	How do you wash pre-cut/packaged lettuce? (check all that apply)	
	Cool tap water (n = 32)	42.7
	Warm tap water (n = 2)	2.7
	Hot tap water (n = 0)	0
	Use some type of vegetable wash (n = 1)	1.3
9.	How do you store pre-cut/packaged lettuce after you open the bag? (n = 77)	
	Leave in original bag	59.7
	Transfer to closed container	7.8
	Transfer to resealable bag	32.5
10.	Where do you store pre-cut/packaged lettuce after you open the bag? (n = 77)	
	Vegetable drawer	75.3
	In refrigerator body	24.7
11.	How long do you store pre-cut/packaged lettuce in the refrigerator? (n = 77)	
	Less than 2 days	6.5
	2 to 4 days	36.4
	5 to 7 days	45.4
	7 to 10 days	11.7
	More than 10 days	0
12.	In general, does the pre-cut/packaged lettuce you store at home stay fresh until you eat it? (n = 77)	
	Yes	63.6
	No	36.4
13.	How do you decide if pre-cut/packaged lettuce is no longer edible? (check all that apply)	
	Appearance (defects, color, etc.) (n = 72)	93.5
	Texture (wilting) (n = 57)	74.0
	Length of storage (n = 21)	27.3
	Odor (n = 36)	46.8
	Other (expiration date on bag, taste) (n = 2)	2.6

Table 10. *Continued*

Questions	Percentage
14. When you eat lettuce, which attributes are important to you? (check all that apply)	
Flavor (n = 63)	81.8
Aroma (n = 29)	37.7
Crispness (texture) (n = 72)	93.5
Appearance (n = 73)	94.8
Other (nutritional content) (n =2)	2.6
15. Many outbreaks of foodborne illness caused by harmful microorganisms have been associated with eating fresh lettuce (n =76).	
True	25.0
False	22.4
Don't know	52.6
16. Have you been sick during the last 12 months from eating lettuce purchased from the grocery store? (n = 77)	
Yes	1.3
No	93.5
Don't know	5.2
17. Are you concerned about the possibility of becoming sick from eating lettuce?	(n = 77)
Yes	22.1
No	77.9
18. Would you expect pre-cut/packaged lettuce treated with an antibacterial solution to stay fresh longer than non-treated lettuce? (n = 77)	
Yes	63.6
No	36.4
19. If yes, how much longer would you expect pre-cut/packaged lettuce treated with an antibacterial solution to stay fresh at refrigeration temperature? (n = 43)	
1 to 7 days	69.8
8 to 14 days	30.2
15 to 21 days	0
20. Would you be willing to buy pre-cut/packaged lettuce already treated at the packinghouse/processing plant with an antibacterial solution? (n = 76)	
Yes	75.0
No	25.0

Table 10. *Continued*

Questions		Percentage
21.	How much more would you be willing to pay for pretreated lettuce? (n = 48)	
	5 to 10 cents more per bag	62.5
	11 to 15 cents more per bag	10.4
	16 to 20 cents more per bag	16.7
	21 to 25 cents more per bag	8.3
	More than 25 cents per bag	2.1

Iceberg was the variety purchased most often (81.1%) and 54% purchased lettuce at least once a week. About 55% purchased whole head lettuce most often while 44% purchased the pre-cut/packaged form. About 45% ate lettuce once or twice per week, and 42% ate it more often (at least 3 to 4 times per week).

More than half (52.6%) of the consumers did not wash pre-cut/packaged lettuce before using it while 47.4% did. After opening the bag, 59.7% stored pre-cut lettuce in the original container, 32.5% transferred it to a resealable bag, and 7.8% transferred it to a closed container. Three-fourths stored pre-cut/packaged lettuce in the vegetable drawer rather than in the body of the refrigerator (on shelf). For those who washed pre-cut/packaged lettuce, cool tap water was used by more consumers than warm tap water or some type of vegetable wash; none used hot tap water. Nearly all of the consumers (94.6%) drained pre-cut/lettuce after washing; drain time of 10 minutes or less was specified by 83.4% of the respondents.

About 45% of the consumers stored pre-cut/packaged lettuce in the refrigerator for 5 to 7 days, 36.4% stored for 2 to 4 days, and 11.7% for 7 to 10 days. About 64% felt that pre-cut/ packaged lettuce stored at home stayed fresh until it was eaten. Of the criteria consumers used to decide if pre-cut/packaged

lettuce was no longer edible, appearance (defects, color) was the most important (93.5%) followed by texture or wilting (74.0%), odor (46.8%), and length of storage (27.3%); a few respondents (2.6%) mentioned other criteria such as expiration date and taste. Quality characteristics of lettuce that were important to consumers were appearance (94.8%), followed by crispness (93.5%), flavor (81.8%), and aroma (37.7%); nutrition/health benefit was mentioned by a few respondents (2.6%).

In response to the question about associating fresh lettuce consumption with outbreaks of foodborne illness caused by harmful microorganisms, 52.6% indicated they did not know. Most (93.5%) indicated they had not been sick during the last 12 months from eating lettuce, and 78% were not concerned about the possibility of becoming sick from eating lettuce.

About 64% of the participants would expect pre-cut/packaged lettuce treated with an antibacterial solution to stay fresh longer than untreated lettuce. Of those who expected extended shelf life of treated lettuce, 69.8% expected 1 to 7 days and 30.2% expected 8 to 14 days; none expected 15 to 21 days. Three-fourths of the respondents would be willing to buy pre-cut/packaged lettuce that had already been treated at the packinghouse/processing plant with an antibacterial solution. Of those who would buy pretreated lettuce, 62.5% would be willing to pay 5 to 10 cents more per bag, 16.7% would pay 16 to 20 cents more, 10.4% would pay 11 to 15 cents more, 8.3% would pay 21 to 25 cents more; only 2% would be willing to pay more than 25 cents.

As demonstrated above, the present invention increases the microbiological safety of a food product, such as lettuce. In addition, lettuce treated in accordance

with the present invention compared favorably in sensory quality to untreated lettuce after 3 days of refrigerated storage. After 10 days, lettuce treated in accordance with the present invention was rated equal to or better than untreated lettuce, depending upon the green color of the leaves. After 15 days, lettuce treated in accordance with the present invention was superior in sensory quality compared to untreated, provided that the lettuce had considerable green color initially.

While the invention has been illustrated and described in detail in the drawings and foregoing description, such illustration and description is to be considered as exemplary and not restrictive in character, it being understood that only a preferred embodiment has been shown and described and that all changes and modifications that come within the spirit of the invention are desired to be protected.

What is claimed is:

1. A method of preparing a food product, comprising:

(a) contacting said food product with an aqueous solution containing about 2% hydrogen peroxide for a time period of about 60 seconds or greater, wherein the aqueous solution has a temperature of about 50°C.

2. The method of claim 1, wherein:

said time period of (a) is between about 60 seconds and about 90 seconds.

3. The method of claim 1, further comprising:

(b) placing said food product into a void defined by a wall of a container.

4. The method of claim 3, further comprising:

(c) allowing a gas to be advanced through said wall of said container.

5. The method of claim 4, wherein:

said gas includes oxygen.

6. The method of claim 1, further comprising:

(d) cooling said food product to about 4°C after (a).

7. The method of claim 1, wherein:

said food product includes a vegetable.

8. The method of claim 7, wherein:

said vegetable is a member of Asteraceae.

9. The method of claim 1, wherein:

said food product is uncooked.

10. A method for treating a vegetable, comprising:

(a) contacting said vegetable with a solution containing about 2% hydrogen peroxide for a time period of about 60 seconds to about 90 seconds, wherein the aqueous solution has a temperature of about 50°C.

11. The method of claim 10, further comprising:

(b) cutting said vegetable into a number of pieces.

12. The method of claim 10, wherein:

said vegetable is selected from the group consisting of Asparagus lettuce, Bibb lettuce, Boston lettuce, Butterhead lettuce, Celtuce, Cos, Crisphead lettuce, Curled lettuce, Garden lettuce, Green oak-leaf lettuce, Green romaine lettuce, Head lettuce, Iceberg lettuce, Lettuce, Limestone lettuce, Lolla rossa, Loose-leaf lettuce, Perella Red, Red oak-leaf lettuce, Red romaine lettuce, Romaine lettuce, and Tango lettuce.

13. The method of claim 11, further comprising:

(c) placing said food product into a void defined by a wall of a container, wherein said wall of said container is permeable to a gas.

14. The method of claim 10, further comprising:

(d) cooling said food product to about 4°C after (a).

15. A method of treating lettuce, comprising:

(a) providing an aqueous solution which has a temperature of about 50°C and includes about 2% hydrogen peroxide; and

(b) placing said lettuce in contact with said aqueous solution for a time period of about 60 seconds to about 90 seconds.

16. The method of claim 15, further comprising:

(c) cutting said lettuce into a number of pieces.

17. The method of claim 16, further comprising:

(d) placing said lettuce product into a void defined by a wall of a container, wherein said wall of said container is permeable to oxygen.

18. The method of claim 17, further comprising:

(e) cooling said lettuce to about 4°C.

19. A method of treating lettuce, comprising:

(a) placing said lettuce in contact with a solution (i) having a temperature of about 50°C and (ii) consisting essentially of water and about 2% hydrogen peroxide; and

(b) placing said lettuce in contact with said solution for a time period of about 60 seconds or greater.

20. The method of claim 19, wherein:

said time period of (b) is between about 60 seconds and about 90 seconds.

21. The method of claim 20, further comprising:

(c) cutting said lettuce into a number of pieces.

22. The method of claim 21, further comprising:

(d) placing said lettuce product into a void defined by a wall of a container, wherein said wall of said container is permeable to oxygen.

23. The method of claim 22, further comprising:

(e) cooling said lettuce to about 4°C.

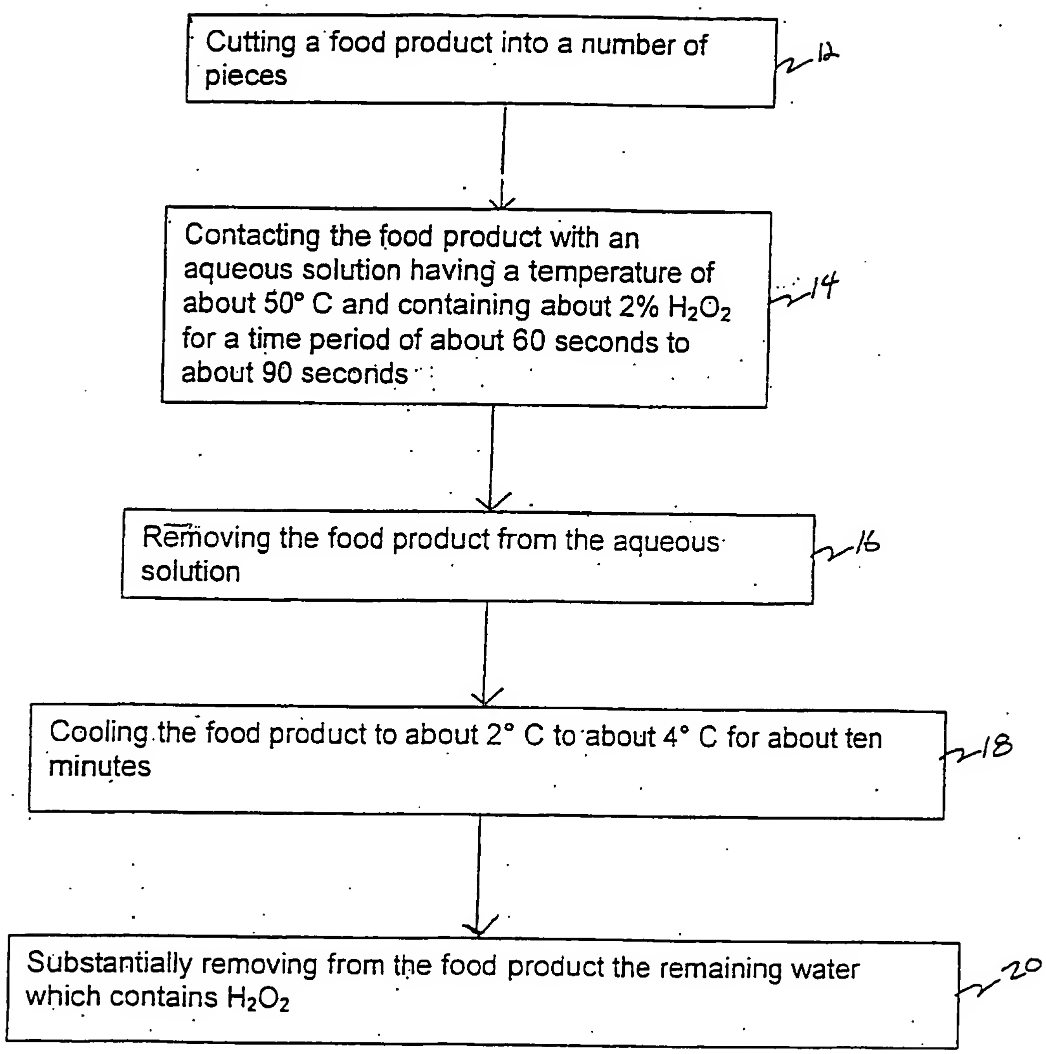

Cutting a food product into a number of pieces
12
Contacting the food product with an aqueous solution having a temperature of about 50° C and containing about 2% H_2O_2 for a time period of about 60 seconds to about 90 seconds
14
Removing the food product from the aqueous solution
16
Cooling the food product to about 2° C to about 4° C for about ten minutes
18
Substantially removing from the food product the remaining water which contains H_2O_2
20

FIG. 1

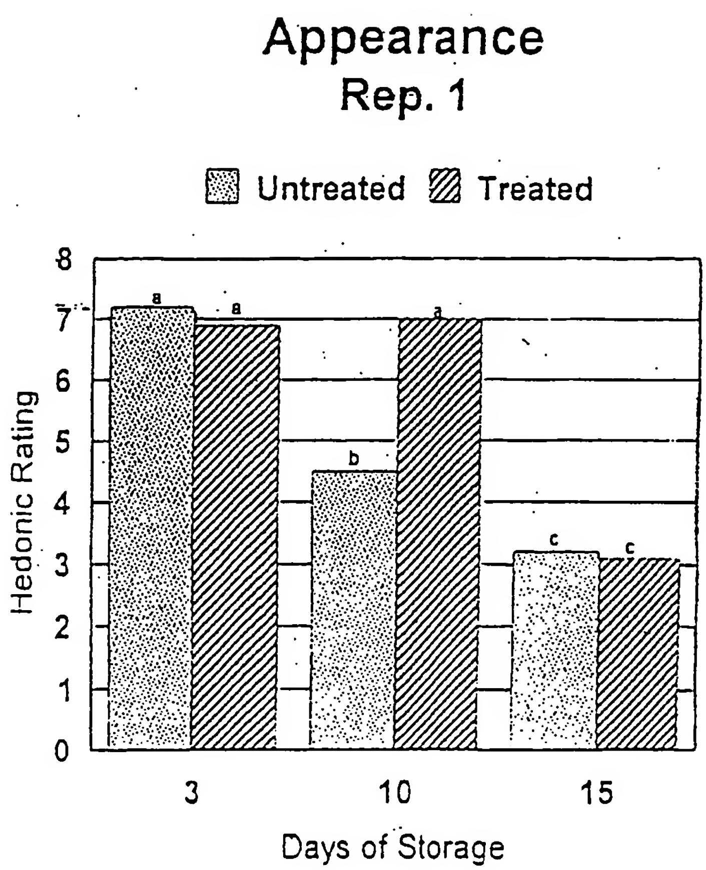
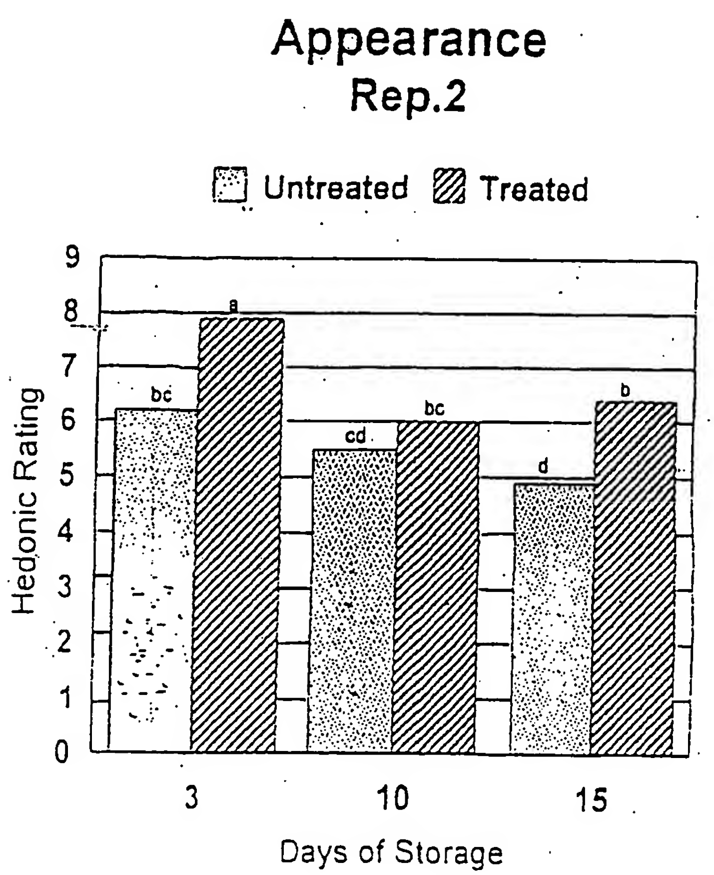

Appearance
Rep. 1
Untreated
Treated
a
a
a
b
c
c
8
7
6
5
4
3
2
1
0
Hedonic Rating
3
10
15
Days of Storage

FIG. 2



Appearance
Rep.2
Untreated
Treated
Hedonic Rating
9
8
7
6
5
4
3
2
1
0
bc
a
cd
bc
d
b
3
10
15
Days of Storage

FIG. 3

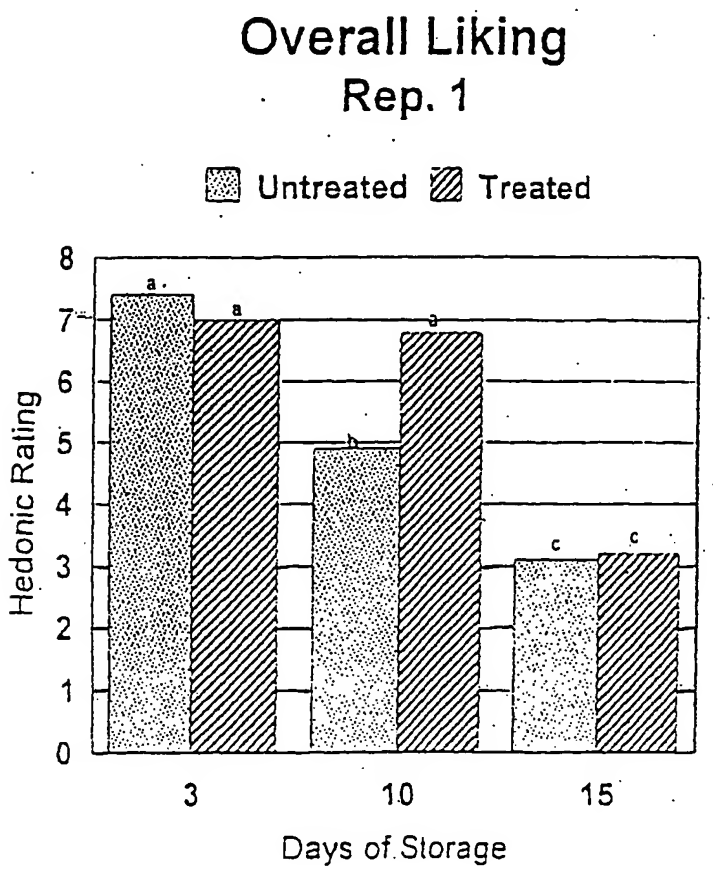

Overall Liking
Rep. 1
Untreated
Treated
Hedonic Rating
8
7
6
5
4
3
2
1
0
a
a
b
a
c
c
3
10
15
Days of Storage

FIG. 4

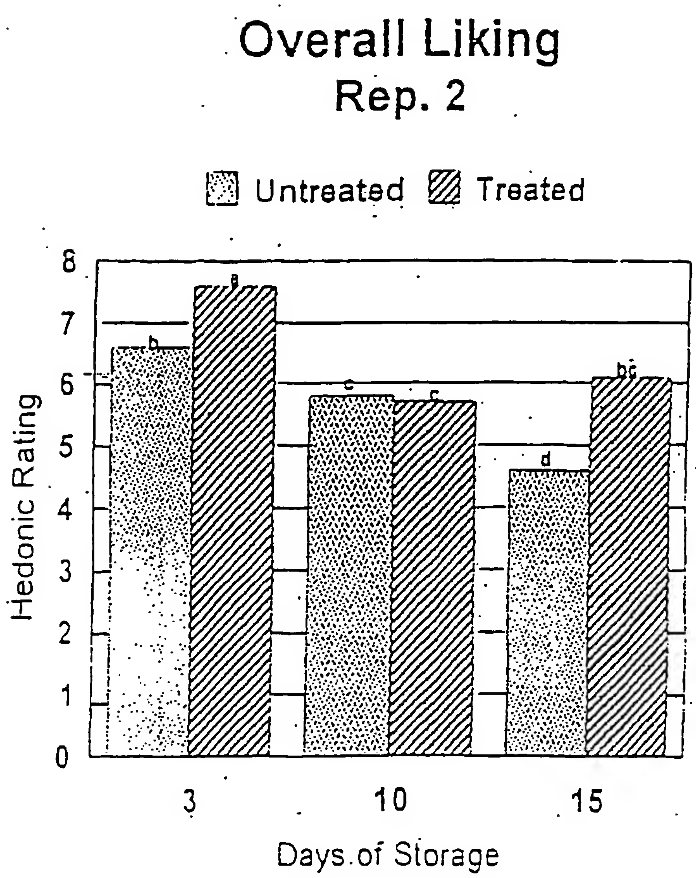

Overall Liking
Rep. 2
Untreated
Treated
Hedonic Rating
0
1
2
3
4
5
6
7
8
b
a
c
c
d
bc
3
10
15
Days of Storage

FIG. 5

FIG. 6

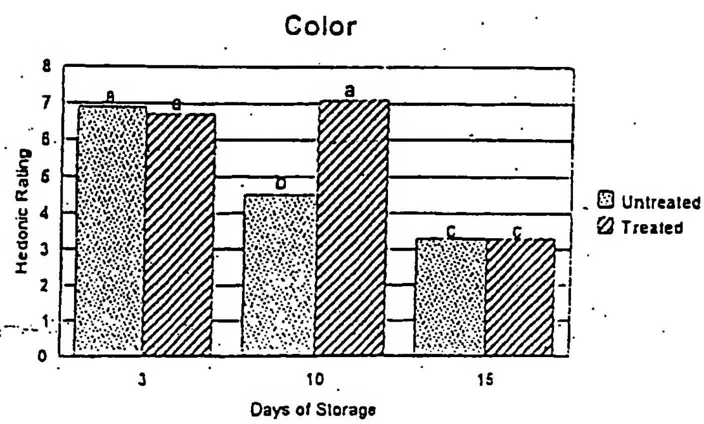

Color
Hedonic Rating
0
1
2
3
4
5
6
7
8
a
a
b
a
c
c
3
10
15
Days of Storage
Untreated
Treated

FIG. 7

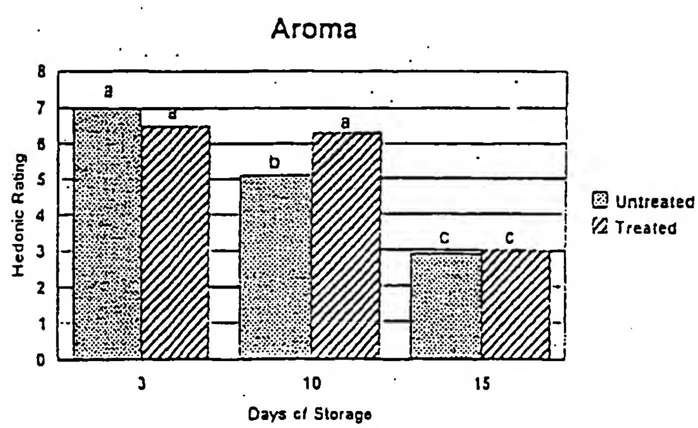

Aroma
Hedonic Rating
0
1
2
3
4
5
6
7
8
a
a
b
a
c
c
3
10
15
Days of Storage
Untreated
Treated

FIG. 8

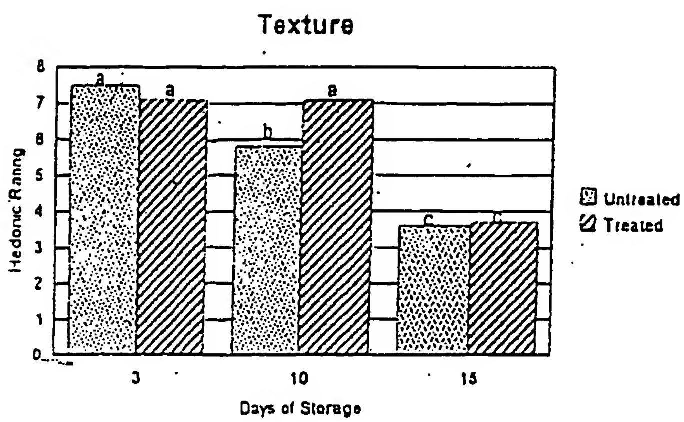

Texture
Hedonic Rating
Days of Storage
Untreated
Treated
3
10
15

FIG. 9

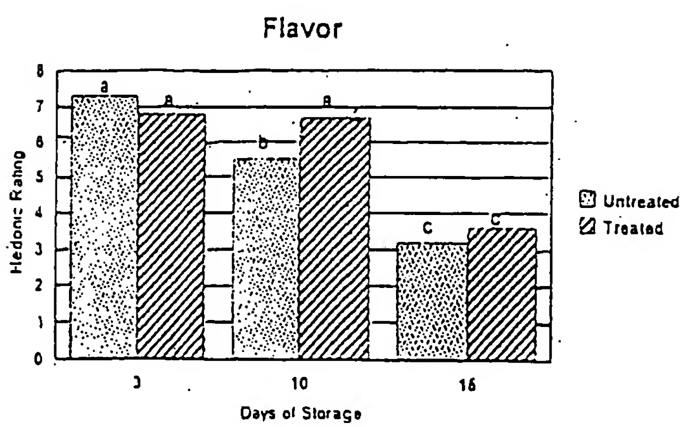

Flavor
Hedonic Rating
Days of Storage
Untreated
Treated
3
10
15

Fig. 10

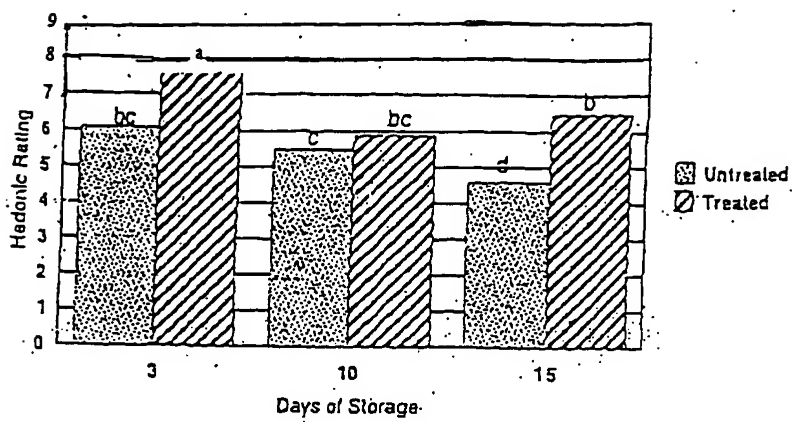
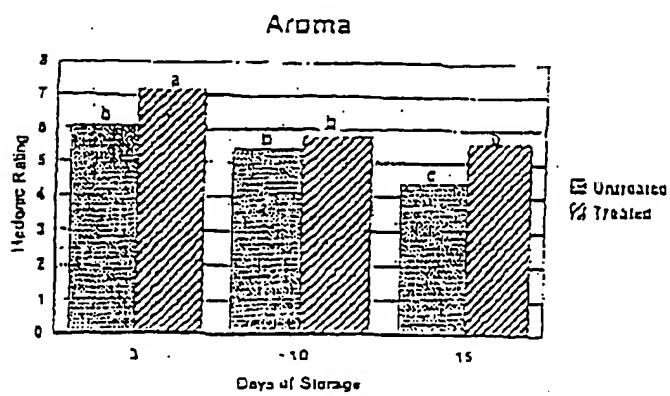

Hedonic Rating
9
8
7
6
5
4
3
2
1
0
a
bc
c
bc
d
b
3
10
15
Days of Storage
Untreated
Treated

FIG. 11


Aroma
Hedonic Rating
7
6
5
4
3
2
1
0
a
b
b
b
c
10
15
Days of Storage

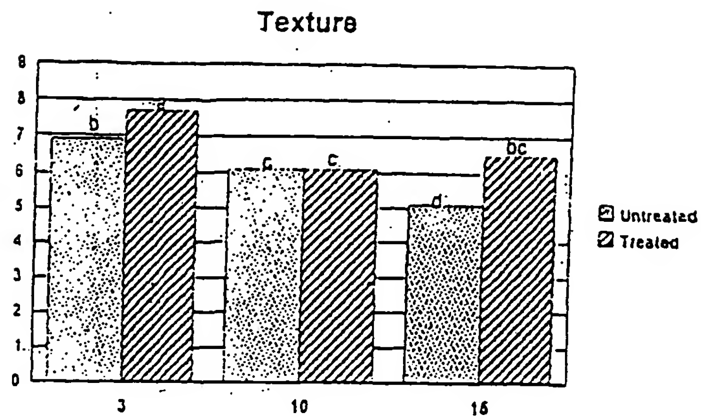

Texture
b
a
c
c
d
bc
Untreated
Treated
3
10
16

FIG. 12

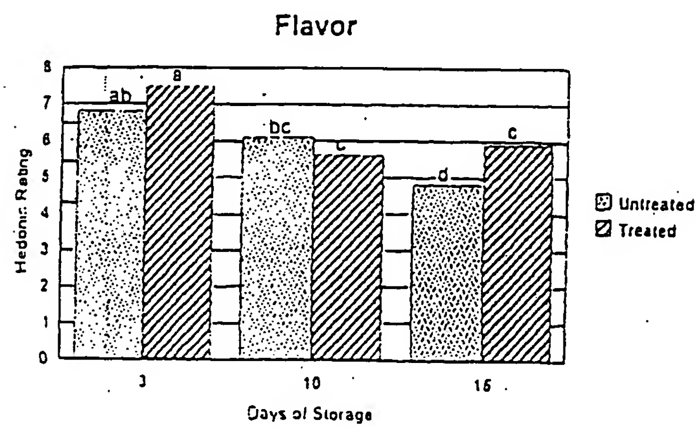

Flavor
Hedonic Rating
ab
a
bc
c
d
c
Untreated
Treated
3
10
16
Days of Storage

FIG. 13

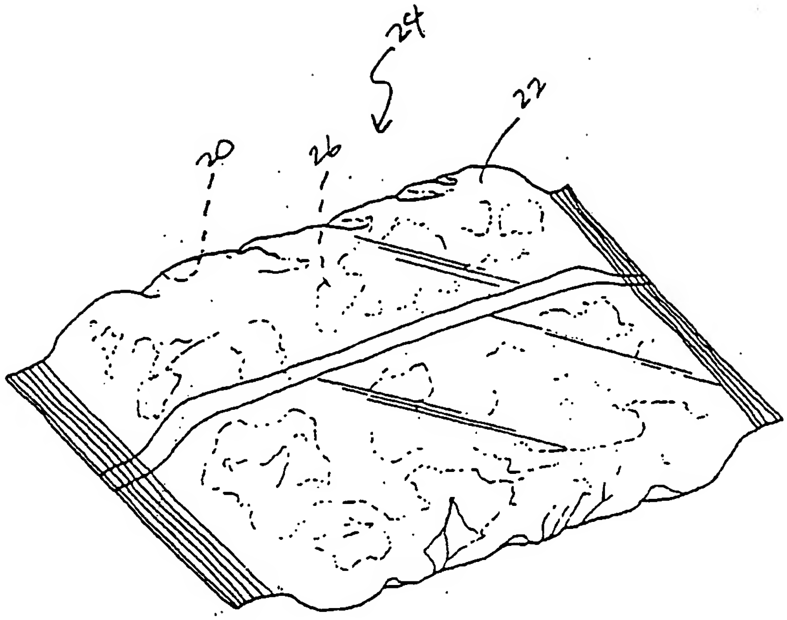


FIG. 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/04097

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A23B 7/153
US CL : 426/335
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 426/335

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,810,512A (KRATKY ET AL) 07 MARCH 1989 (07.03.89), See entire document.	1-23
Y	WO 96/21360 (INNOVEST AG) 18 JULY 1996 (18.07.96), see abstract	1-23
Y	WO 94/15475 (WU ET AL) 21 JULY 1994 (21.07.94), see entire document.	1-23
Y	US 4,224,347 A (WOODRUFF) 23 SEPTEMBER 1980 (23.09.80), see entire document.	1-23
Y	US 4,943,440 A (ARMSTRONG) 24 JULY 1990 (24.07.90), see entire document.	1-23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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